

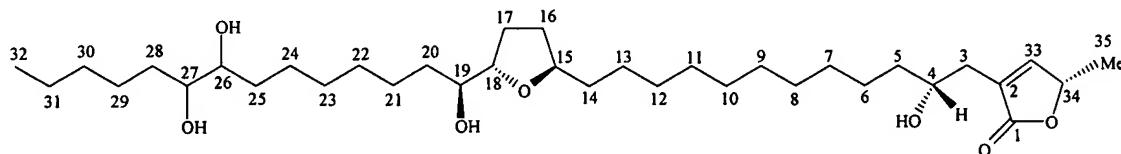
Yang-Chang WU
Application No. 10/005,324
Amdt. dated November 9, 2004
Reply to Office action of August 9, 2004

This listing of claims will replace all prior versions, and listings, of claims in the application:

Listing of Claims:

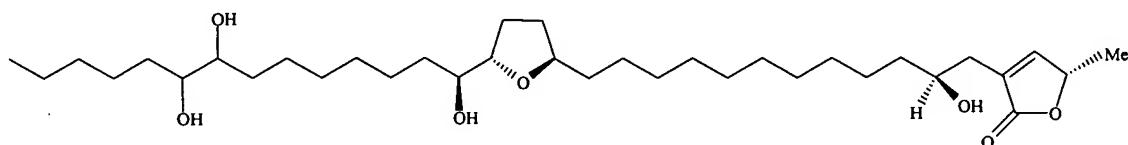
Claim 1 (previously amended). Isolated and purified Annonaceous acetogenin compounds having the structures of a-g, wherein

a. muricin A has the formula of:



said muricin A having an α , β -unsaturated γ -lactone with a hydroxyl group at C-4, a mono-THF ring placed between C-15 and C-18 with one flanking hydroxyl in a threo conformation, two methylene groups of the mono-THF ring corresponding to a trans conformation, two hydroxyl groups at C-26 and C-27 as vicinal diol assigned as threo based, and the stereochemistry at C-34 on the γ -lactone fragment performed in (S)-configuration;

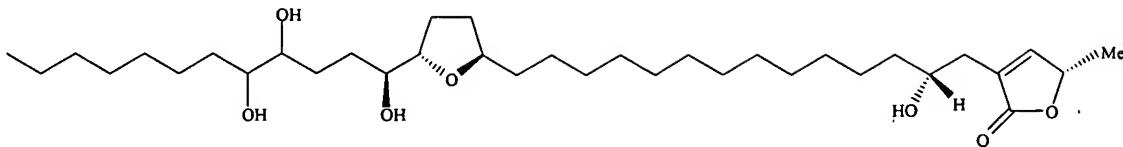
b. muricin B has the formula of:



said muricin B having an α , β -unsaturated γ -lactone with a hydroxyl group at C-4, a mono-THF ring placed between C-15 and C-18 with one flanking hydroxyl in a trans/threo conformation, two methylene groups of the mono-THF ring corresponding to a trans conformation, two hydroxyl groups at C-26 and C-27 as vicinal diol assigned as threo based, and the stereochemistry at C-34 on the γ -lactone fragment performed in (S)-configuration;

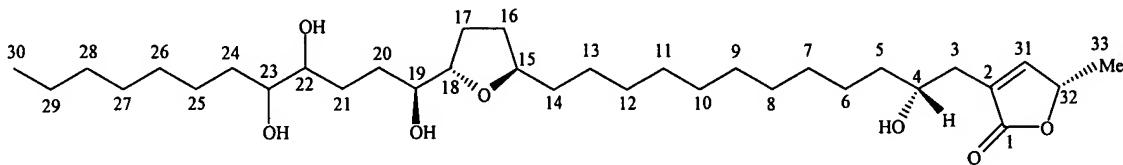
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c. muricin C has the formula of:



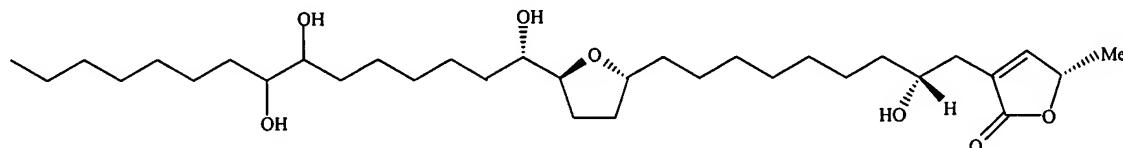
said muricin C having an α , β -unsaturated γ -lactone with a hydroxyl group at C-4, a mono-THF ring placed between C-17 and C-20 with one flanking hydroxyl in a trans/threo or threo/trans conformation, two hydroxyl groups at C-24 and C-25 as vicinal diol assigned as threo based, and the stereochemistry at C-34 on the γ -lactone fragment performed in (S)-configuration;

d. muricin D has the formula of:



said muricin D having an α , β -unsaturated γ -lactone with a hydroxyl group at C-4, a mono-THF ring placed between C-15 and C-18 with one flanking hydroxyl in a threo/trans conformation, two hydroxyl groups at C-22 and C-23 as vicinal diol assigned as threo based;

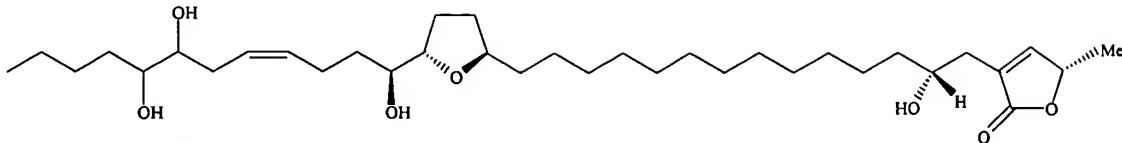
e. muricin E has the formula of:



said muricin E having an α , β -unsaturated γ -lactone with a hydroxyl group at C-4, a mono-THF ring placed between C-12 and C-15 with one flanking hydroxyl in a threo/trans conformation, two hydroxyl groups at C-22 and C-23 as vicinal diol assigned as threo based;

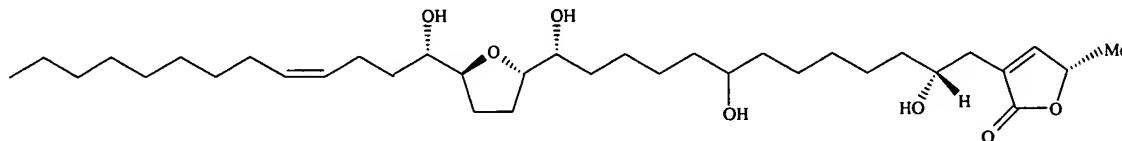
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f. muricin F has the formula of:



said muricin F having an α, β -unsaturated γ -lactone with a hydroxyl group at C-4, a mono-THF ring placed between C-17 and C-20 with one flanking hydroxyl in a threo/trans conformation, two hydroxyl groups at C-27 and C-28 as vicinal diol assigned as threo based, and a double bond determined at C-24/C-25; and

g. muricin G has the formula of:



Claim 2 (currently amended) A method for isolating ~~and purifying~~ the said

Annonaceous acetogenins compounds according to claim 1 comprising:

extracting said Annonaceous acetogenins compounds ~~muricins~~ from *Annona muricata* seeds with MeOH to obtain a MeOH extract at room. temperature; and

evaporating said MeOH from said MeOH extract; and

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partitioning said evaporated the MeOH extract in a CHC₁₃ and aqueous mixture, whereby said Annonaceous acetogenins compounds are in said the CHC₁₃ layer of said the CHC₁₃ and aqueous mixture.

Claims 3-4 (cancelled).

Claim 5 (currently amended). A pharmaceutical ~~An anti-tumor~~ composition comprising an amount of substantially pure muricins of claim 1, wherein the muricins are selected from the group consisting of muricin A, muricin B, muricin C, muricin D, muricin E, muricin F, and muricin G; [.] and wherein the muricins are ~~effective and act as an anti-tumor agent and~~ combined with a pharmaceutically acceptable carrier in said the ~~anti-tumor~~ composition.

Claim 6 (currently amended). The pharmaceutical composition ~~Annonaceous acetogenins compounds~~ as claimed in claim 5 4, wherein said pharmaceutical composition is cytotoxic to human cancer cells ~~the Annonaceous acetogenins compounds are used for treatment of patients having a tumor.~~

Claim 7 (currently amended). The pharmaceutical ~~anti-tumor~~ composition as claimed in claim 5 6, wherein said human cancer cells are the anti-tumor composition is used for pharmaceutically treating a patient having hepatoma cancer cells.

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Claim 8 (currently amended). A method for ~~of~~ treating a patient having a tumor, wherein said method comprising the step of:

administering an effective amount of said a pharmaceutical composition according to claim 5 comprising muricins of claim 1 to a said patient having a tumor.

Claim 9 (currently amended). A method for treating a patient with hepatoma ~~cancer~~ comprising administering to a said patient ~~afflicted~~ with hepatoma ~~cancer~~ an effective amount of said a pharmaceutical composition ~~comprising at least one Annonaceous acetogenins compounds according to claim 5~~ ~~and a pharmaceutically acceptable salt and ester in combination with pharmaceutically acceptable carrier, auxiliary or excipient.~~

Claim 10 (previously added). The isolated and purified Annonaceous acetogenins compounds according to claim 1, wherein said compound is isolated from *Annona muricata*.

Claim 11 (previously added). The isolated and purified Annonaceous acetogenins compounds according to claim 10, wherein said compound is isolated from seeds of *Annona muricata*.

Claims 12-15 (cancelled).

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Claim 16 (currently amended). The method according to claim 15 19, wherein the muricin A, muricin B, muricin C, and muricin F are eluted from the seventh fraction of the Si gel column and further purified by a reversed-phase high performance liquid chromatography.

Claim 17 (currently amended). The method according to claim 15 19, wherein the muricin D (4), muricin E (5), and muricin G (7) are eluted from the eighth fraction of the Si gel column and further purified by a reversed-phase high performance liquid chromatography.

Claim 18 (cancelled). The anti-tumor composition according to claim 5, wherein said composition further comprises a pharmaceutically acceptable salt and/or ester in combination with a pharmaceutically acceptable carrier, auxiliary or excipient.

Claim 19 (new). A method for isolating and purifying said Annonaceous acetogenins compounds according to claim 1, comprising:

extracting said Annonaceous acetogenins compounds from *Annona muricata* seeds with MeOH to obtain an MeOH extract at room. temperature; and
evaporating said MeOH from said MeOH extract;
partitioning said evaporated MeOH extract in a CHCl₃ and aqueous mixture to separate said evaporated MeOH extract into a CHCl₃ layer and an aqueous layer;
collecting said CHCl₃ layer;

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loading said CHCl₃ layer onto an Si gel column and eluting said isolated and purified said Annonaceous acetogenins compounds from said Si gel column with a gradient containing n-hexane-CHCl₃ and CHCl₃-MeOH into 10 fractions.

Claim 21 (new). The method according to claim 16, wherein said reversed-phased HPLC is an ODS-5 column with MeOH-water at a volume ratio of about 88:12.

Claim 22 (new). The method according to claim 17, wherein said reversed-phased HPLC is an ODS-5 column with MeOH-water at a volume ratio of about 86:14.